Foliar micromorphology and anatomy of *Ugni molinae* Turcz. (Myrtaceae), with particular reference to schizogenous secretory cavities.

Hernan A. Retamales[A] , Rosa Scherson[B] , Tanya Scharaschkin[A]

[A]School of Earth, Environmental and Biological Sciences, Science and Engineering Faculty. Queensland University of Technology. Brisbane, QLD 4001, Australia.

[B]Plant Biology Laboratory, Faculty of Forest Sciences and Nature Conservation, University of Chile, P.O. Box 9206, Santiago, Chile.

Corresponding Author: hernanalfonso.retamales@student.qut.edu.au

Abstract

Background: *Ugni molinae* Turcz. is one of the most studied species of South American Myrtaceae due to its edible fruits and foliar medicinal compounds. However, there is no anatomical study of the leaves or secretory cavities. This paper seeks to describe the leaf micromorphology and anatomy of the species using standard protocols for light and scanning electron microscopy. Secretory cavities were anatomically characterized in young and mature leaves. Histochemical staining of the cavities was performed.

Results: The leaves of *Ugni molinae* are hypostomatic, have a wavy surface and possess scattered hairs. Leaf anatomical features include dorsiventral mesophyll, 2-3 layers of palisade parenchyma with abundant chloroplasts, calcium oxalate crystals and internal phloem in vascular bundles. Schizogenous secretory cavities are present on the abaxial surface and are mainly located on the margins of the leaves. Histochemical tests of these cavities suggest the presence of lipophilic substances.

Conclusions: This is the first study of secretory cavities in Chilean Myrtaceae. In general, micromorphological and anatomical characters are similar to other species of the family. The present findings could provide valuable anatomical information for future research in South American Myrtaceae.

Keywords: Anatomy; Myrtaceae; secretory cavities; SEM; terpenoids

Background

Myrtaceae Juss. (Myrtales; APGIII 2009) is a large family of angiosperms including approximately 5500 species, divided into two subfamilies, 17 tribes and ca. 140 genera (Wilson et al. 2005; Biffin et al. 2010). It is a predominantly southern hemisphere family with a high diversity in South America and Australasia (Snow 2000). The genus *Ugni* Turcz.

comprises four species distributed in South America from the Andean region from Chile to Mexico (Wilson 2011). *Ugni molinae* Turcz (Myrtaceae) is a South American shrub that occurs in the humid temperate forests of south-central Chile and Argentina (Figure 1 A) (Landrum 1988). The species is known as "murta", "murtilla" or "Chilean guava" due to its edible fruits (Aguirre et al. 2006). Other commercial products include tea, essential oils and alcoholic extracts (Landrum 1988; Quilaqueo et al. 2012). The leaves and fruits of *U. molinae* are rich in antioxidants and phytoestrogenic substances that are used to treat digestive disorders, inflammations, urinary infections and diabetes (Rubilar et al. 2011; Avello et al. 2013). A number of studies have investigated the chemical compounds and the biochemical activity in leaves of *U. molinae*, identifying polyphenols (condensed tannins), terpenoids, flavonoids and nanocomposite films of carboxymethylcellulose (Aguirre et al. 2006; Rubilar et al. 2006; Avello et al. 2013; Doll et al. 2012; Quilaqueo et al. 2012). The other three species of *Ugni*, namely *U. candollei* (continental Chile), *U. selkirkii* (Juan Fernandez Archipielago) and *U. myricoides* (Mexico, Central America to Bolivia) are not considered equally important in economic terms as *U. molinae* (Wilson 2011).

Secretory cavities are a common feature in Myrtaceae (Metcalfe and Chalk 1979), but there are few studies regarding anatomy of these structures in the family. Chemicals produced by secretory cavities also need more investigation, as not many genera have been anatomically described (P.G. Wilson, pers. comm.). Similarly, development of secretory cavities in Myrtaceae has been scarcely investigated (Cicarelli et al. 2003). Although leaf chemistry of *U. molinae* has been comprehensively studied, there is no information regarding its leaf anatomy and micromorphology. Since leaf anatomy is unknown, anatomical structure and development of secretory cavities in the species remain without clarification. The aims of this paper are (1) to describe the surface micromorphology and internal anatomy of leaves of *U.*

molinae for the first time and (2) to investigate the anatomical structure of secretory cavities in early and mature stages.

Methods

Sampling

Fresh leaf material was collected from natural populations in Chile. Mature leaves were collected randomly from sun-exposed branches in different individuals from Futrono, Región de los Ríos (40° 7' 28" S / 72° 22' 51" W) and Talcahuano, Región del Bio-Bio (36°43'0"S / 73°7'0"W). Young leaves were also collected as trichomes or certain structures are reported to be early caduceus (Landrum 1988). Fresh leaves were fixed in FAA for 24-48h. Specimens (Reta-04.1, Reta-04.2, Reta-04.3 and Reta-04.4) are currently housed at the Queensland Herbarium (BRI), Brisbane, Australia. Duplicates (Reta-04.5, Reta-04.6) are housed at EIF (Forestry Sciences, Universidad de Chile).

Scanning electron microscopy (SEM)

Fixed leaf material was dehydrated using a graded ethanol series and then critical point dried (Anderson 1951) in an Autosamdri-815 automatic critical point drier. Samples were mounted on stubs with self-adhesive double-sided carbon discs and sputter-coated with gold palladium for 175 sec using a Leica EM SCD005 Gold Coater. Examination and photography were conducted using a FEI Quanta 200 SEM/ESEM operated at 10kV.

Light microscopy

Fixed material was dehydrated through a graded ethanol series, infiltrated and embedded in paraffin wax (Johansen 1940; Ruzin 1999). Transverse sections were cut using a Leica RM2245 rotary microtome at 5µm and placed onto microscope slides. Sections were stained

using a 0.05% (w/v) of ruthenium red in distilled water for 2 minutes and counterstained with a solution 0.1% (w/v) of toluidine blue (TBO) in distilled water for 45 sec. Sections were dehydrated post-staining through a graded ethanol series and mounted using Depex. Additional histochemical tests were performed using freehand sections of fixed leaves (Cutler et al. 2008) and stained with phloroglucinol-HCl, Sudan IV and IKI to test the composition of secreted chemicals if secretory cavities are present in the species. Leaf clearings were prepared by immersing tissue fragments of 1-2 cm^2 in 10% KOH at room temperature for 48 h followed by 7% NaClO for 2 h or until leaves turned transparent. Cleared leaves were washed five times with distilled water, stained with Safranin O/TBO and mounted with lactoglycerol (lactic acid-glycerol 1:1). Slides were observed using a Nikon SMZ 800 Stereo light microscope (Nikon eclipse 50i compound) and images captured using the Nikon NIS-Elements imaging software.

Terminology

Terminology for describing Myrtaceae leaf micromorphology was based on previous descriptions in van Wyk et al. (1982), Fontenelle et al. (1994) and Haron and Moore (1996). Terminology for leaf anatomy was based on Schmid (1980), Schmid and Baas (1984), Keating (1984), Cardoso *et al.* (2009) and Soh and Parnell (2011). Other general references consulted for anatomical terminology were Esau (1953), Gifford and Foster (1989), Dickison (2000) and Cutler et al. (2008).

Results

Leaf overview

The leaves of *U. molinae* are simple, opposite and lanceolata, elliptic or ovate in shape. The apex is acuminate and the base acute to rounded. The blades have a rough and uneven

appearance on the adaxial surface (Figure 1 B). Leaves are dorsiventral and have a noticeable depression directly above the midrib (Figure 2 A). Leaves have numerous secondary and tertiary reticulate veins. Venation is pinnate and weakly to strongly brochidodromus (Figure 2 B). Venation is barely visible externally.

Cuticle, epidermal cells and stomata

The adaxial surface has a prominent cuticle, which probably contains polyphenols due to bluish-green staining with TBO. On this surface, the cuticle has a wavy and undulate deposition and might be the reason of the rough appearance of leaves (Figure 2 C). Adaxial and abaxial epidermal cells are compressed, plano-convex, mainly isodiametric and containing abundant tannins (Figure 2 C). In paradermal view, adaxial epidermal cells have straight cell walls and are very compressed, while abaxial epidermal cells are irregularly rounded and the anticlinal cell walls are strongly sinuous (Figure 2 D). Leaves of *U. molinae* are hypostomatic and stomatal complexes are anomocytic (Figure 2 D). Stomata are circular to elliptical and are located at the same level as the epidermal cells. The dimension of stomata is 10–15 μm long. Guard cells are kidney-shaped and the cell lumen between them is narrow at the equatorial region. Guard cells have cutinized thickenings on the outer periclinal walls, which are more visible in cross section (Figure 2 E). Stomata are regularly distributed on the surface of the leaf (Figure 3 A).

Trichomes

Adaxial surface of leaves is glabrous (Figure 3 B), while abaxially have some scattered hairs, which are sparsely strigose on the midrib. Hairs are simple, unicellular, non-glandular, solitary, conical and slightly wavy. Both deciduous and persistent hairs are present (Figure 3 C). Bases of deciduous trichomes are abundant on the midrib of the abaxial surface.

Midrib

The midrib is strongly impressed on the adaxial surface (Figure 1 B, Figure 3 B) and slightly prominent below. There is a notorious depression on the adaxial surface above the midrib. This species has internal phloem as in other Myrtaceae. The midrib is arc-shaped with a strong curvature (Figure 4 A). There is a strongly developed adaxial phloem partition. Incurved margins of the phloem well developed. Adaxial and abaxial phloem not confluent. Fibres are discontinuous around the midrib. Xylem vessels of the midrib show scalariform perforation plates and helical wall thickenings. Phloem sieve tubes and companion cells have thin primary cell walls. Phloem fibres have evident and thick secondary cell walls.
An extension of the bundle sheath, composed of rounded-polygonal cells, is clearly visible under the midrib and slightly developed above.

Mesophyll

The mesophyll is formed by a 3-4 layered palisade parenchyma and a spongy parenchyma with abundant intercellular spaces (Figure 2 A). The palisade parenchyma layer is somewhat dense and composed of rectangular, attenuated and vertical cells. These cells possess thin primary cell walls and numerous chloroplasts. The spongy parenchyma is composed of irregular shaped cells (rounded to star-shaped). Both palisade and spongy parenchyma contain mucilage. Subepidermal idioblasts with calcium oxalate crystals (druses) occur throughout the palisade parenchyma (Figure 4 B).

Secretory cavities

Leaf secretory cavities are exclusively located under the abaxial epidermis of leaves and some cases as part of this layer. The cavities are slightly visible above the epidermis level and

form a pronounced swelling (Figure 3 D). Secretory cavities are composed of large spaces surrounded by a sheath of peripheral epithelial cells, which are almost disintegrated in mature leaves. These structures are particularly close to the margin of leaves (Figure 4 C). The presence of epithelial cells surrounding a cavity space demonstrates the schizogenous development of the cavities for separation of cells and not dissolution as the lysigenous type. Epithelial cells are initially developed from the protodermis and ground meristem as shown in leaf primordia (Figure 4 D). These cells are small (12 ± 4 μm of diameter), compressed and have very thin cell walls. The final numbers of epithelial cells is reached quickly after the first periclinal divisions in the meristem. During leaf expansion and differentiation, secretory cavities increase their size and the area of the cavity becomes larger (Figure 4 D, E). In mature leaves, these structures are abundant throughout the mesophyll and have variable dimensions (75 ± 35 μm of diameter). Histochemical reaction with Sudan IV reveals the presence of lipophilic substances in the epithelial cells. (Figure 4 D). This was the only positive reaction among the performed histochemical tests.

Discussion

Even though species of Myrtaceae are rich in essential oils and other chemical compounds, information concerning leaf anatomy and histochemistry is scarce. *U. molinae* is one of the most studied species of South American Myrtaceae, but mainly regarding chemical composition. Results of this investigation show that *U. molinae* shares a number of anatomical traits with other species of the family. These characters include druses (calcium oxalate crystals), adaxial phloem and secretory cavities. Calcium oxalate crystals are abundant in the leaves of *U. molinae*, especially in the palisade parenchyma, just below the adaxial epidermis. Druses are widely present in several genera of Myrtaceae, in diverse vegetative and reproductive structures. Donato and Morretes (2007, 2009) and Alves et al.

(2008) described druses of calcium oxalate in South American species of *Eugenia.* Donato and Morretes (2011) reported the same structures for *Myrcia multiflora*. Polyhedral crystals, including druses, have been reported in *Psidium*, *Eugenia*, *Gomidesia* and *Myrcia*, among others (Cardoso et al. 2009; Gomes et al. 2009). The function of these structures is not completely clear, but has been related to the regulation of calcium and other minerals (Volk et al. 2002), as well as protection against herbivores and pathogens (Franceschi and Nakata 2005; Korth et al. 2006). The habitat preference of *U. molinae* supports this hypothesis, due to the species normally occurs in calcium-rich soils and is severely attacked by insects (Kausel 1947; Navas 1980).

Internal phloem was found in midribs, either as continuous tissue or strands in the adaxial side of the midrib. This character is regarded as a typical anatomical character in the order Myrtales (Cronquist 1981; Takhtajan 1980) and is widely present in Myrtaceae (Schmid 1980; Cardoso et al. 2009). The development of the adaxial phloem, confluence between adaxial and abaxial phloem and continuity of fibres around midrib are regarded as suitable characters to identify species in Myrtaceae (Cardoso et al. 2009; Soh and Parnell 2011).

Helical wall thickenings on vessel elements of the xylem tissue have been reported in a number of Myrtaceae genera, such as *Myrceugenia, Myrtus, Austromyrtus, Myrcia, Myrcianthes* and *Psidium* (Schmid and Baas 1984). Similarly, scalariform perforation plates on vessels have been identified in *Myrceugenia, Luma, Tepualia, Ugni, Neomyrtus* and *Myrtastrum* (Schmid and Baas 1984). Helical wall thickenings of vessels and scalariform perforation plates as observed in *U. molinae*, have been attributed to putatively primitive species (Stern 1978). The latter, possibly as an adaptation of a common ancestor to cooler or mountain environments (Jansen et al. 2004).

The secretory cavities follow the typical schizogenous pattern commonly observed in Myrtaceae (Alves et al. 2008; Donato and Morretes 2007; Gomes et al. 2009). The observation of undifferentiated epithelial cells in leaf primordia and developed ones in mature leaves confirms this pattern. Similarly, Cicarelli et al. (2003, 2008) describe this type of initial development of secretory cavities in *Myrtus communis*. These authors report that the ontogeny of secretory cavities in *M. communis* follows an schizolysigenous development, which is a combination of lysigenous (due to disintegration of cells) and schizogenous. Origin of schizogeneous cavities in Myrtaceae has been suggested from protoderm or epidermal meristems, with participation of the ground meristem (Arruda and Fontenelle 1994; Fahn 1979). In *U. molinae*, the origin has been identified from both of these tissues. Secretory cavities are produced by periclinal divisions of these meristems, which produce sets of internal and external cells. Internal cells produce the epithelial cells of the cavities, while the more external remain as epidermal cells. Development of secretory cavities matches with the descriptions of Fahn (1979).

The main chemical compounds identified in leaves of South American Myrtaceae include cyclic sesquiterpenes and monoterpenes in *Blepharocalyx* (Godinho et al. 2014), *Eugenia*, *Myrcia* and *Psidium* (Stefanello et al. 2011). Other compounds are flavonoids (Wollenweber et al. 2000), tannins (Tanaka et al. 1996) and triterpenoids (Lee 1998; Judd et al. 1999). Triterpenoids are widely present in many families of plants and are produced in different parts of the leaves, not only in secretory cavities (Xiao et al. 2008). Histochemical test with Sudan IV suggests that secretory cavities of *U. molinae* produce mainly lipophilic compounds, similar to those identified in South American Myrtaceae. Other tests with phloroglucinol-HCl and IKI were negative in secretory cavities.

Monoterpenes, among the most members are α- *β*-pinene, 1,8-cineole, limonene, sabinene, terpinen-4-ol and *α*-terpineol have been detected in several species of Myrtaceae (Shellie et al. 2004; Stefanello et al. 2011; Victorio et al. 2011). Secretory cavities in *Myrrhinium atropurpureum* have been confirmed as producers of these compounds (Victorio et al. 2011). The role of terpenoids and monoterpenes has been associated to a number of plant functions. These roles are related to direct defense responses (Cheng et al. 2007), metabolism of diverse chemicals (Banthorpe et al. 1972), plant-environment interactions (Lange and Ahkami 2013) and plant architecture, through inhibition of shoot branching (Akiyama et al. 2008).

Conclusion

In this paper, the foliar micromorphology and anatomy of *U. molinae* has been described for the first time. Schizogenous secretory cavities are abundant in leaves and produce mainly lipophilic compounds, according to histochemical staining. This is the first report regarding anatomical structure, development and histochemistry of secretory cavities in the species. The species shares a number of anatomical and micromorphological characters with other Myrtaceae. Results from this investigation are potentially useful for future anatomical studies in South American Myrtaceae.

Competing interests

The authors declare that they have no competing interests (financial or non-financial).

Authors' contributions

HR conceived the study, carried out the anatomical and micromorphological analyses and drafted the manuscript. RS helped with the sampling strategy, academic input in early stages of this project and also provided laboratory supplies for field work. TS was responsable for important academic input for the study, including experimental design and editingof the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was funded by Conicyt-Government of Chile. The authors are very thankful to Helen O'Connor, Rachel Hancock and Amy Carmichael (QUT) for helping with the embedding, sectioning and image acquisition respectively. Thanks to the Plant Structure and Systematics group (QUT) for advice and feedback. We appreciate the valuable feedback from an anonymous reviewer.

References

Aguirre MC, Delporte C, Backhouse N, Erazo S, Letelier ME, Cassels B, Silva X, Alegría S, Negrete R (2006) Topical anti-inflammatory activity of 2a-hydroxy pentacyclic triterpene acids from the leaves of *Ugni molinae*. Bioorganic & Medicinal Chemistry 14:5673–5677.

Akiyama K, Arite T, Hanada A, Kamiva Y, kyouza J, Magome H (2008) Inhibition of shoot branching by new terpenoid plant hormones. Nature 455:195.

Alves E, Tresmondi F, Longui E (2008) Análise estrutural de folhas de *Eugenia uniflora* L. (Myrtaceae) coletadas em ambientes rural e urbano, SP, Brasil. Acta Botanica Brasilica 22(1):241-248.

Anderson T (1951) Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. Transactions of the New York Academy of Sciences 13:130.

Angiosperm Phylogeny Group (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society 161:105-121.

Arruda RCO, Fontenelle GB (1994) Anatomia foliar de *Psdium cattleyanum*. Revista Brasileira de Botanica 17:25-35.

Avello MA, Pastene ER, Bustos ED, Bittner ML, Becerra JA (2013) Variation in phenolic compounds of *Ugni molinae* populations and their potential use as antioxidant supplement. Brazilian Journal of Pharmacognosy 23(1):44-50.

Banthorpe DV, Charlwood BV, Francis MJO (1972) The biosynthesis of monoterpenes. Chemical Reviews 72:115-149.

Biffin E, Lucas E, Craven L, Ribeiro da Costa I, Harrington M, Crisp M (2010) Evolution of exceptional species richness among lineages of fleshy-fruited Myrtaceae. Annals of Botany 106:79-93.

Cardoso CMV, Proença SL, Sajo MG (2009) Foliar anatomy of the subfamily Myrtoideae (Myrtaceae). Australian Journal of Botany 57(2):148-161.

Cheng A, Lou YG, Mao YB, Lu S, Wang LJ, Chen XY (2007) Plant terpenoids: Biosynthesis and ecological functions. Journal of Integrative Plant Biology 49(2):179-186.

Ciccarelli D, Pagni AM, Andreucci AC (2003) Ontogeny of secretory cavities in vegetative parts of *Myrtus communis* L. (Myrtaceae): an example of schizolysigenous development. Israel Journal of Plant Sciences 51:193-198.

Ciccarelli D, Garbari F, Pagni AM (2008) The flower of *Myrtus communis* (Myrtaceae): Secretory structures, unicellular papillae, and their ecological role. Flora - Morphology, Distribution, Functional Ecology of Plants 203(1): 85-93.

Cronquist A (1981) An integrated system of classification of flowering plants. Columbia University Press, New York.

Cutler DF, Botha T, Stevenson DW (2008) Plant anatomy: An applied approach. Oxford Blackwell Publishing, London.

Dickison W (2000) Integrative plant anatomy. Harcourt Academic Press, San Diego.

Doll U, Rodriguez I, Soto C, Razmilic I (2012) Cutting propagation and tannin and flavonoid concentration in leaves of two *Ugni molinae* provenances from the Maule Region (Chile). Bosque 33(2): 203-209. DOI: 10.4067/S0717-92002012000200010.

Donato AM, Morretes BL (2007) Anatomia foliar de *Eugenia brasiliensis* Lam. (Myrtaceae) proveniente de áreas de restinga e de floresta. Revista Brasileira de Farmacognosia 17: 426-443.

Donato AM, Morretes BL (2009) Anatomia foliar de *Eugenia florida* DC. (Myrtaceae). Revista Brasileira de Farmacognosia 19:759-770.

Donato A, Morretes B (2011) Leaf morphoanatomy of *Myrcia multiflora* (Lam.) DC.-Myrtaceae. Revista Brasileira de Plantas Medicinais 13(1): 43-51.

Esau K (1953) Plant anatomy. John Wiley and Sons Inc, New York.

Fahn A (1979) Secretory tissues in plants. Academy Press, London.

Fontenelle GB, Costa CG, Machado RD (1994) Foliar anatomy and micromorphology of eleven species of *Eugenia* L. (Myrtaceae). Botanical Journal of the Linnean Society 116(2):111-133.

Franceschi V, Nakata P (2005) Calcium oxalate in plants: Formation and function. Annual Review of Plant Biology 56:41-71.

Gifford E, Foster A (1989) Morphology and evolution of vascular plants. Third edition. Freeman, New York.

Godinho W, Farnez M, Pereira I, Regorio L, Grael C (2014) Volatile constituens from leaves of Blepharocalyx salicifolius (Kunth) O.Berg (Myrtaceae). Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 13(3):249-253.

Gomes SM, Somavilla N, Gomes-Bezerra KM, Miranda S, De-Carvalho PS, Graciano-Ribeiro D (2009) Anatomia foliar de espécies de Myrtaceae: contribuições à taxonomia e filogenia. Acta Botanica Brasilica 23(1):223-238.

Haron NW, Moore DM (1996) The taxonomic significance of leaf micromorphology in the genus *Eugenia* L. (Myrtaceae). Botanical Journal of the Linnean Society 120:265–277.

Jansen S, Baas P, Gasson P, Lens F, Smets E (2004) Variation in xylem structure from tropics to tundra: evidence from vestured pits. Proceedings of the National Academy of Sciences of the United States of America 101:8833-8837.

Johansen DA (1940) Plant microtechnique. Mc Graw Hill, London.

Judd W, Campbell CS, Kellogg EA, Stevens PF (1999) Plant systematics: a phylogenetic approach. Sinauer Associates, Sunderland.

Kausel E (1947) Notas mirtológicas. Lilloa 11(4):320-327.

Keating RC (1984) Leaf histology and its contribution to relationships in the Myrtales. Annals of the Missouri Botanical Garden 71:801-823.

Korth K, Doege S, Park S, Goggin F, Wang Q, Gomez S, Liu G, Jia L, Nakata P (2006) *Medicago truncatula* mutants demonstrate the role of plant calcium oxalate crystals as an effective defense against chewing insects. Plant Physiology 141:188-195.

Landrum LR (1988) The myrtle family (Myrtaceae) in Chile. Proceedings of the California Academy of Sciences 45:277-317.

Lange BM, Ahkami A (2013) Metabolic engineering of plant monoterpenes, sesquiterpenes and diterpenes—current status and future opportunities. Plant Biotechnology Journal 11:169–196.

Lee C (1998) Ursane triterpenoids from leaves of *Melaleuca leucadendron*. Phytochemistry 49:1119-1122.

Metcalfe C, Chalk L (1979) Anatomy of the dicotyledons. Clarendron Press, Oxford.

Navas E (1971) Distribución geográfica de las Mirtáceas Chilenas. Boletín del Museo Nacional de Historia Natural 29:223-247.

Quilaqueo M, Echeverria I, Ihl M, Bifani V, Mauri A (2012) Carboxymethylcellulose–montmorillonite nanocomposite films activated with murta (*Ugni molinae* Turcz.) leaves extract. Carbohydrate Polymers 87:1495– 1502.

Rubilar M, Pinelo M, Ihl M, Schuermann E, Sineiro J, Nuñez MJ (2006) Murta leaves (*Ugni molinae* Turcz.) as a source of antioxidant polyphenols. Journal of Agricultural and Food Chemistry 54:59-64 59.

Rubilar M, Jara C, Poo Y, Acevedo F, Gutierrez C, Sineiro J, Shene C (2011) Extracts of maqui (*Aristotelia chilensis*) and murta (*Ugni molinae* Turcz.): Sources of antioxidant compounds and r-glucosidase/r-amylase Inhibitors. Journal of Agricultural and Food Chemistry 59:1630-1637. dx.doi.org/10.1021/jf103461k.

Ruzin SE (1999) Plant microtechnique and microscopy. Oxford University Press, New York.

Schmid R (1980) Comparative anatomy and morphology of *Psiloxylon* and *Heteropyxis,* and the subfamilial and tribal classification of Myrtaceae. Taxon 29(5/6):559-595.

Schmid R, Baas P (1984) The occurrence of scalariform perforation plates and helical vessel wall thickenings in wood of Myrtaceae. IAWA Bulletin 5:197-215.

Shellie R, Mondello L, Dugo G, Marriott P (2004) Enantioselective gas chromatographic analysis of monoterpenes in essential oils of the family *Myrtaceae.* Flavour and Fragrance Journal 19(6):582-585.

Snow N (2000) Systematic conspectus of Australasian Myrtinae (Myrtaceae). Kew Bulletin 55:647-654.

Soh W, Parnell J (2011) Comparative leaf anatomy and phylogeny of *Syzygium* Gaertn. Plant Systematics and Evolution 297(1-2):1-32.

Stefanello M, Pascoal A, Salvador M (2011) Essential oils from neotropical Myrtaceae: Chemical diversity and biological properties. Chemistry & Biodiversity 8:73-94.

Stern W (1978) A retrospective view of comparative anatomy, phylogeny and plant taxonomy. IAWA Bulletin 2:33-39.

Takhtajan AL (1980) Outline of the classification of flowering plants (Magnoliophyta). The Botanical Review 46(3):225-359.

Tanaka T, Orii Y, Nonaka G, Nishioka I, Kuono I (1996) Syzyginins A and B, two ellagitannins from *Syzygium aromaticum.* Phytochemistry 43:1345-1348.

Van Wyk A, Robbertse PJ, Kok P (1982) The genus *Eugenia* L. (Myrtaceae) in southern Africa: The structure and taxonomic value of stomata. Botanical Journal of the Linnean Society 84:41-56.

Victorio CP, Moreira CB, Souza MD, Sato A (2011) Secretory cavities and volatiles of *Myrrhinium atropurpureum* Schott *var. atropurpureum* (Myrtaceae): An endemic species collected in the restingas of Rio de Janeiro, Brazil. Natural Product Communications 6(7):1045-1050.

Volk G, Lynch-Holm V, Kostman T, Goss L, Franceschi V (2002) The role of druse and raphide calcium oxalate crystals in tissue calcium regulation in *Pistia stratiotes* leaves. Plant Biology 4:34-45.

Wilson P, O'Brien M, Heslewood M, Quinn C (2005) Relationships within Myrtaceae *sensu lato* based on a *mat*K phylogeny. Plant Systematic and Evolution 251:3-19.

Wilson P (2011) Myrtaceae. In: Kubitzki K (eds) The families and genera of vascular plants. Vol. X. Flowering plants Eudicots: Sapindales, Cucurbitales, Myrtaceae. Springer-Verlag, Heidelberg, pp 212–271.

Wollenweber E, Wehde R, Dörr M, Lang G, Stevens J (2000) C-Methyl-flavonoids from the leaf waxes of some Myrtaceae. Phytochemistry 55:965-970.

Xiao W, Li R, Huang S, Pu J, Sun H (2008) Triterpenoids from the Schisandraceae family. Natural Product Reports 25(5):871-891. dx.doi.org/10.1039/b719905h

Figure 1. *Ugni molinae*, habit and leaf morphology. A, Shrub. B, Leaves.

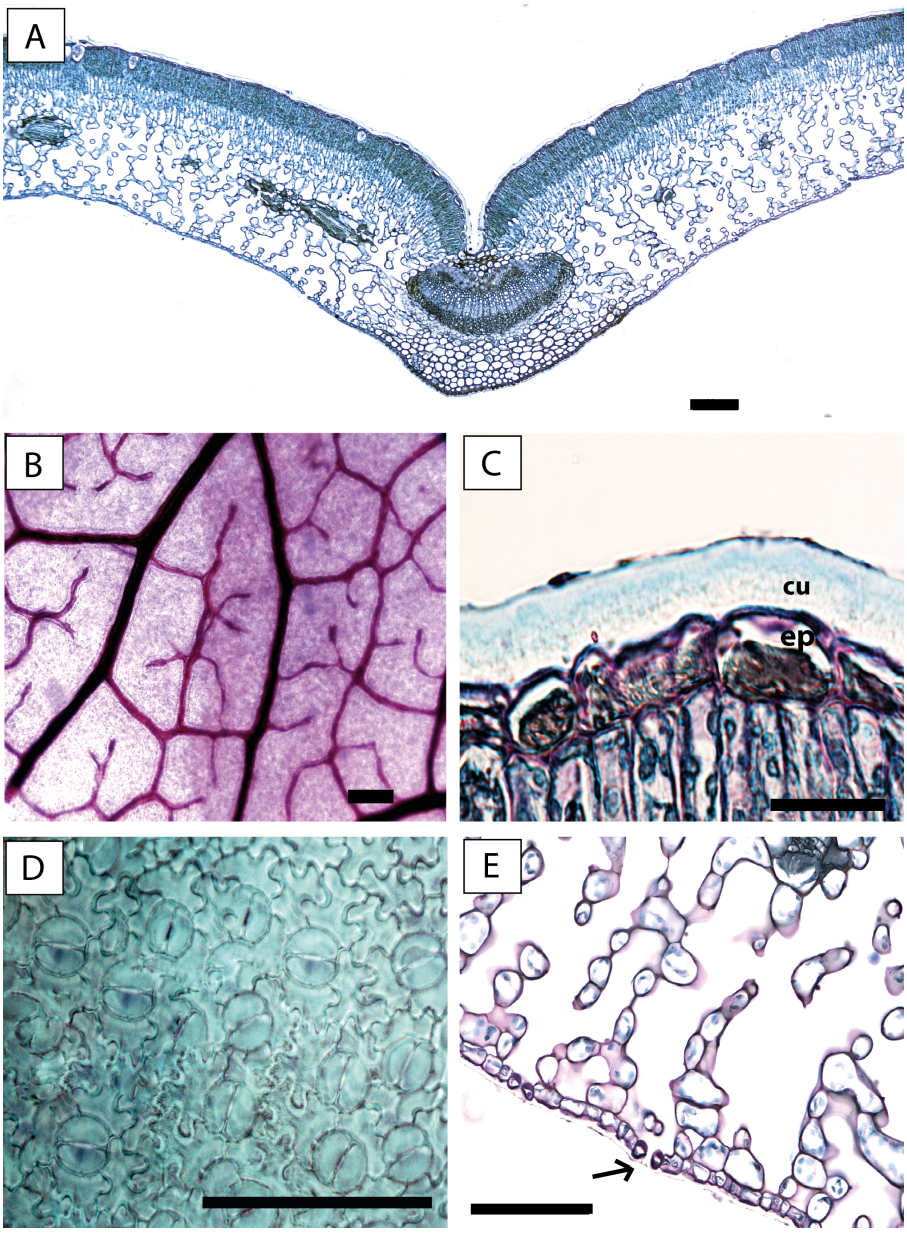

Figure 2. Light micrographs (LM) of the leaves of *U. molinae*. A, Anatomical overview of the leaf blade. B, Leaf clearing showing reticulate tertiary venation. C. Epidermal cells and cuticle. D, Leaf clearing showing sinuous epidermal cells and anomocytic stomata on the abaxial surface. E, Transverse section of the mesophyll showing stomata with cutinized outer periclinal walls of guard cells and stomatal chamber connected to the spongy parenchyma. **cu-** cuticle, **ep-** epidermal cells. Arrow: stomata. Scale bars = 100 µm.

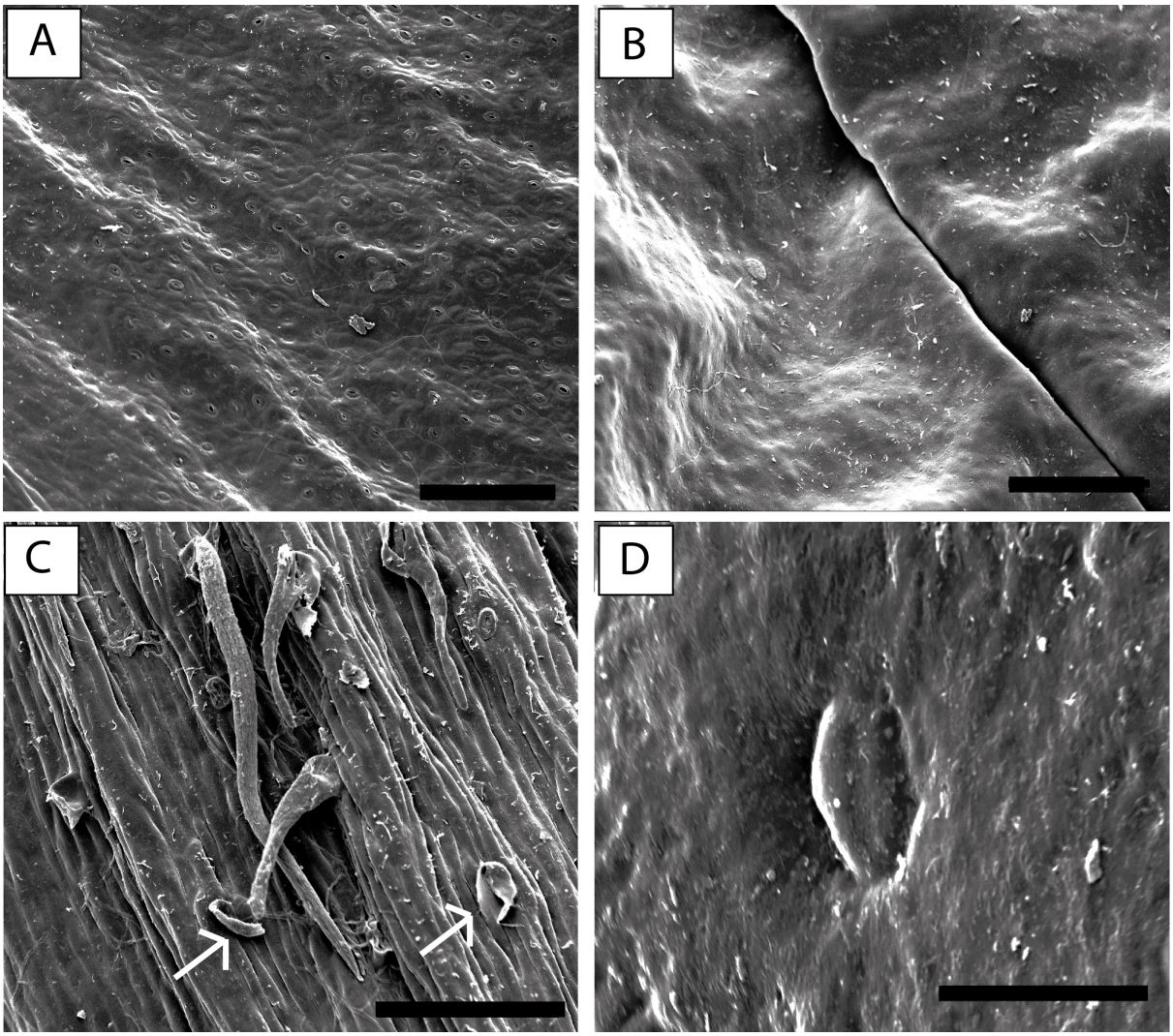

Figure 3. Scanning electron micrographs (SEM) of leaves of *U. molinae*. A, Abaxial surface showing stomatal distribution. B, Adaxial surface showing impressed midrib and glabrescent wavy surface. C, Unicellular hairs and bases of deciduous trichomes on the abaxial midrib (arrows). D, SEM micrograph of subepidermal secretory cavity, visible as a pronounced swelling. Scale bars A, B= 100 μm; C, D= 50 μm.

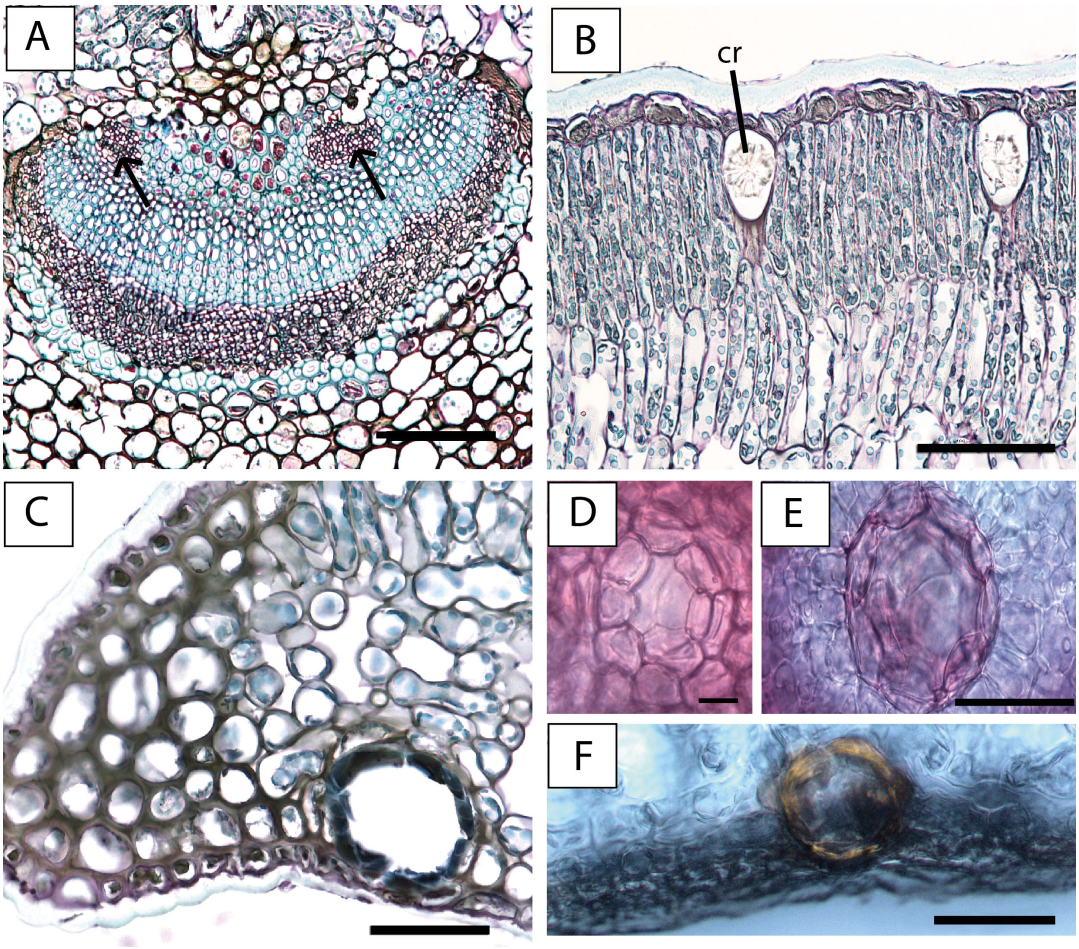

Figure 4. Light micrographs (LM) showing midrib, mesophyll and secretory cavities of *U. molinae*. A, Detail of midrib showing deoiveloped adaxial phoem partition, incurved margins of the phloem and discontinuous fibres. B, Transverse section of the leaf showing cells with idioblasts containing druses (calcium oxalate crystals) below the epidermis. C, Transverse section of leaf showing a secretory cavity and epithelial cells on the abaxial surface close to the leaf margin. D, Early developmental stage of secretory cavity showing epithelial cells in formation. E, Secretory cavity with eight epithelial cells in formation in young leaves. F, Free-hand section showing secrektory cavity on the abaxial side of the leaf. Histochemical reaction indicates lipophilic compounds in the epithelial cells after testing with Sudan IV (orange colour). **cr-** crystals (druses). Arrows: internal phloem. Scale bars A-C, E-F = 100 µm, D=10 µm.